Evaluation of a Mobile Two-Stage Decontamination System using a Power Washer Combined with Eight Disinfectant Treatments

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Abstract: With the increased frequency of pandemics that threaten the spread of zoonotic diseases associated with agricultural commodities and trade, it is becoming a national priority to advance more effective and efficient decontamination technologies. A field study was conducted to evaluate a two-stage, mobile power washing system. The study factors were power washing, disinfectant type, sample surfaces, and number of repeat disinfectant applications. Study factors were evaluated based on log10 reduction of viable *Bacillus subtilis* spores on inoculated sample surfaces. Diluted bleach from Clorox Concentrate, applied without power washing, had the greatest sporicidal activity when applied three times to non-porous surfaces (steel washers), which resulted in a 3.0 log10 reduction of viable spores. The two-stage decontamination treatment with the greatest sporicidal activity was power washing porous surfaces (wool fabric), followed by three applications of EasyDECON DF-200, resulting in a 4.8 log10 reduction of viable spores. The results showed that power washing was the most important factor for dislodging spores and overall decontamination effectiveness. Also, sporicidal activity was slightly greater for non-porous surfaces compared to porous surfaces. Repeated applications of disinfectants resulted in little to no improvement in sporicidal efficacy. The results from this field study were comparable with a similar two-stage equipment decontamination study, which was conducted the year before this study. Further research is needed to evaluate large stationary decontamination systems and refine any interactions between power washing parameters and innovative sanitation methods.

Keywords: Equipment decontamination, Two-stage decontamination, Power washing, Disinfectants.

1. INTRODUCTION

With more than 10.6 million tons per year of freight entering and leaving the United States airports, and 25 million sea containers entering USA ports decontamination of transport containers, transport vehicles, and storage facilities is an increasing concern [1]. Effective decontamination of these sea containers, field equipment, vehicles, and storage systems necessitate a combination of decontamination methods such as power washing followed by disinfectant treatments. Power washing is often used in order to remove and dislodge high levels of grime from contaminated surfaces [2,3]. Power washing is also an effective means of removing spores from treated surfaces in combination with disinfectants [4]. Multi-stage decontamination systems and methods often provide higher sanitation levels that are required for high-risk foreign pests that potentially threaten both agricultural crops and animals in the United States.

Two-stage decontamination consists of power washing equipment followed by disinfectant applications. Two previous studies evaluated the effects of a mobile, two-stage decontamination system, using *Bacillus subtilis* spores as a surrogate microbe, in multi-factor field studies [5,6]. In addition, three other studies have shown that power washing dislodges most pathogens on highly soiled surfaces [2-4]. Extending the disinfectant contact time becomes critical during low humidity conditions, which often occur within the summer months in the western region of the United States. A field study, involving low humidity conditions, found that disinfectant contact times may drop below one minute for a single spray application [6]. Repeated disinfectant applications may extend the exposure time under low humidity conditions and increase the overall efficacy of the spray applications.

In this study, *B. subtilis* was used as a microbial surrogate to evaluate the effectiveness of each stage in the decontamination process. *B. subtilis* is a gram-positive, bacterium that is commonly found in the soil, air, and plant compost [7]. The endospore stage allows it to survive under the harshest environmental conditions, which makes it an excellent surrogate for field studies [8,9].

The study objectives were to determine the sporicidal efficacy of eight disinfectants, power

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washing, and the number of repeat disinfectant applications on porous and non-porous sample surfaces inoculated with *B. subtilis* spores. The results from this study conducted in 2017 were compared to a similar study conducted in 2016 to validate the two studies.

2. MATERIALS AND METHODS

The field study was conducted in 2017 at the Colorado State University Agricultural Research Development and Education Center (ARDEC) near Fort Collins, CO, USA. The study design included four factors, which were fully crossed with each other. The four factors were: two inoculated sample surfaces (porous and non-porous), power washing, eight disinfectants, and repeated disinfectant applications (1, 2, or 3 applications).

All *B. subtilis* spore samples were prepared by a private microbiology laboratory (MicroChem Laboratory, Round Rock, TX, USA). The *B. subtilis* spore and vegetative cell suspensions were treated with isopropanol in order to destroy any vegetative cells, so that only spores remained in the final suspension. For the non-porous surface, steel washers (5 cm) were inoculated with 300 µl of spores, then air-dried to bind the spores to the washer surface. For the porous surfaces, fabric samples, composed of 35% wool and 65% nylon (2.5 x 7.6 cm) were inoculated with 300 µl of *B. subtilis* spores, along the bottom 5 cm of each strip. The fabric samples were used to simulate a vehicle seat cover. Spore inoculation rates were 10^6 and 10^8 Colony Forming Units (CFU)/ml for the steel washer and fabric samples, respectively. All samples were shipped in insulated boxes and stored at 4 C. Samples intended for percent recovery analysis were inoculated and assayed at the microbiology laboratory. In addition, control samples for transport and storage effects on spore viability were shipped and stored along with the treatment samples. Upon completion of the experiment, all treated samples were returned to cold storage (4 C) until they were returned to the microbiology laboratory [10]. The private laboratory assayed the samples for viable *B. subtilis* spores on semi-selective media in order to enumerate the CFU counts for all control and treated samples.

The four study factors in this study were similar to the factors tested in the equipment decontamination study conducted the previous year. The two sample surfaces were porous (wool/nylon fabric) and non-porous (steel washers). Two-stage decontamination was evaluated with and without power washing, i.e. disinfectants applied after power washing (yes), or disinfectants were applied without power washing (no). The eight commercial disinfectants tested were: Intervention (Virox, Oakville, ON, Canada), diluted bleach from Clorox Concentrate (8.5%) (Clorox, Oakland, CA, USA), DioxiGuard (Frontier Pharmaceuticals, Melville, NY, USA), EasyDECON DF-200 (Intelagard, Lafayette, CO, USA), ElectroBiocide [EB (Strategic Resource Optimization LLC, Denver, CO, USA)], Virkon-S (LANXESS Corp. Suffolk, UK), Z-series (ICA Trinova, Newnan, GA, USA), and Sanidate 2.0 (BioSafe Systems LLC, East Hartford, CT, USA). Intervention is an accelerated formulation of hydrogen peroxide (H_2O_2). Clorox Concentrate (8.5%) is a sodium hypochlorite (NaClO) formulation. DioxiGuard and ElectroBiocide are ready-to-use chlorine dioxide (ClO_2) formulations with added surfactants. EasyDECON DF-200 is a pre-mix of hydrogen peroxide and quaternary ammonium formulation. Virkon-S tablets dissolve into hypochlorous acid (HOCl) when mixed with water. The Z-series granules react together to generate a liquid chlorine dioxide (ClO_2) solution

Table 1: Average pH and Oxidation Reduction Potential (ORP) for the Eight Disinfectants Tested for Sporicidal Activity on *B. subtilis* Spores. The Diluted Concentration for each Disinfectant is also Listed

Disinfectant	Diluted concentration (mg/L)	pH	ORP (mV)
Intervention	2,600	1.5	720
Clorox Conc.	7,860	11.6	706
DioxiGuard	160	3.0	935
ElectroBiocide	200	5.5	872
Sanidate 2.0	227	1.5	698
Virkon-S	10,000	2.6	1092
Z-series	250	5.9	1002
DF-200	54,782	8.9	323

when mixed with water [11]. The pH and oxidation reduction potential (ORP) of each disinfectant was measured using a multi-meter (Orion 3 Star, ThermoScientific, Waltham, MA, USA). The final, diluted disinfectant concentration, pH, and ORP for the eight disinfectants are listed in Table **1**. The disinfectants were applied either one, two, or three times per sample.

Steel washer and fabric strips were labeled and attached to the frame of a chisel plow to simulate the decontamination of farm implements. Neodymium magnets (0.6 x 2.5 x 5 cm) (K&J Magnetics, Pipersville, PA, USA) were used to hold the samples to the frame. All samples were placed vertically on the chisel plow frame so any excess disinfectant could drain off. Samples were placed approximately 25 cm apart in order to minimize disinfectant drift onto adjacent samples.

The mobile power washer was manufactured by S-K Environmental (Okanogan, WA, USA). A single wand with a nozzle pressure of 13.8 MPa was used to wash samples at 10 s/sample holding the nozzle approximately 10 cm from the sample surface. The mobile power washer had a single wand water use rate of 15.1 L/min. (4 GPM).

After the samples were power washed and allowed to dry, the disinfectant treatments were applied with a hand spray bottle (Double Mist Trigger Sprayer, Kwazar, UK) at a rate of approximately 4 ml/sample. The two-stage decontamination sequence included power washing for 10 s, air dry for two minutes, application of the disinfectant treatment, and then repeated two or three more times. The samples with only one spray application were neutralized after a ten-minute drying period. The ten-minute disinfectant neutralization time was based on the labels of most of the EPA registered disinfectants that require a ten-minute disinfectant contact time to achieve the stated sanitation levels. The samples with two spray applications, had a two-minute drying period between sprays, followed by an eight-minute drying period before neutralizing. Finally, those samples with three spray applications had a two-minute drying period between sprays that were followed by a six-minute drying until neutralization of the sample. The total disinfectant exposure time (approximately 10 min) for each sample was the same for each spray and disinfectant treatment combination, in order to minimize any effects due to extended exposure times for the second and third repeat applications.

The disinfectant treated samples were neutralized using either sodium thiosulfate (Fisher Scientific, Pittsburgh, PA, USA) or D/E neutralizing broth (Hardy Diagnostics, Santa Maria, CA, USA). Sodium thiosulfate (25g/100 ml H_2O) was used to neutralize the ElectroBiocide, Virkon-S, DioxiGuard, Intervention, and Z-series treated samples. The D/E neutralizing broth (34g broth, 5 ml of Polysorbate 80, and 1,000 ml H_2O) was used to neutralize the Clorox Concentrate, DF-200, and Virkon-S samples. Both neutralizers were applied using a hand spray bottle (approx. 5 ml/sample). Samples were air-dried, labeled, placed in individually labeled pre-sterilized Whirl-Paks (Nasco, Fort Atkinson, WI, USA), and placed in cold storage (4 C).

The study design was developed with JMP software (SAS Institute Inc., Clary, NC, USA), using the Design of Experiment (DOE) program to reduce the number of samples. The DOE design used hidden replication by limiting interaction terms to two-way interactions. By limiting the interaction terms to two-way interactions there were 59 statistical replications for each treatment. Results were significant at α = 0.05. The JMP Least Squares Fit model was used to analyze the log 10 reduction data.

Viable spore counts per sample [CFU/sample] were transformed into log10 reduction values using the following formula [12]:

$$Log10\ Reduction = Log\left(A/B\right)$$

where A was the median number of viable spores recovered from control samples, and B was the number of variable spores recovered from the treated sample.

3. RESULTS

The data presented in Table **1** showed that Clorox Concentrate had the highest pH of 11.59, and Intervention and Sanidate 2.0 shared the lowest pH of 1.5. Virkon-S had the highest ORP of 1,092.4 mV, and EasyDECON DF-200 had the lowest ORP of 323 mV (Table **1**).

All study factors and two, two-way interactions were significant, i.e. sample surface type, power washing, disinfectant, and the number of disinfectant applications affected the *B. subtilis* viable spore log10 reduction results (Table **2**). Although all the study factors influenced sporicidal efficacy, the order of treatment effectiveness, based on F ratios, was as

Table 2: Least Squares Fit Model *p*-Values for Log10 Reduction of Viable *B. subtilis* Spores for Two-Stage Study Factors

Source	F ratio	Prob > F
Power Washing	152.2	<0.0001
Disinfectant	40.8	<0.0001
No. of Disinfectant Applications	12.1	<0.0001
Sample Surface	9.3	0.0028
Power Washing* Disinfectant	10.2	<0.0001
Disinfectant * Sample Surface	4.5	0.0001

Table 3: Log10 Viable Spore Reduction Estimates, Based on the Least Squares Fit Model, for *B. subtilis* Spores Inoculated on Non-Porous Surfaces (Steel Washers) and Treated with Disinfectants

Disinfectant	Power wash (yes or no)	One disinfectant application[a]	Two disinfectant applications	Three disinfectant applications
		Log10 reduction		
Clorox Conc.	No	1.9 (1.4 - 2.4)	2.6 (2.1 - 3.2)	3.0 (2.4 - 3.5)
Dioxiguard	No	0.9 (0.4 - 1.4)	1.6 (1.1 - 2.2)	2.0 (1.5 - 2.5)
ElectroBio	No	0.7 (0.2 - 1.2)	1.5 (0.9 - 2.0)	1.8 (1.3 - 2.3)
Intervention	No	0.6 (0.01 - 1.0)	1.3 (0.8 - 1.8)	1.6 (1.2 - 2.1)
Sanidate	No	1.6 (1.1 - 2.0)	2.3 (1.8 - 2.8)	2.6 (2.2 - 3.1)
Virkon-S	No	1.3 (0.8 - 1.8)	2.0 (1.5 - 2.6)	2.4 (1.9 - 2.9)
Z-series	No	0.4 (-0.2 - 1.0)	1.1 (0.6 - 1.7)	1.5 (0.9 - 2.1)
Clorox Conc.	Yes	4.0 (3.5 - 4.5)	4.1 (3.6 - 4.6)	4.1 (3.5 - 4.6)
DF-200[b]	Yes	4.0 (3.5 - 4.5)	4.1 (3.6 - 4.6)	4.1 (3.6 - 4.6)
Dioxiguard	Yes	3.0 (2.4 - 3.5)	3.1 (2.6 - 3.6)	3.1 (2.5 - 3.6)
ElectroBio	Yes	3.0 (2.3 - 3.6)	3.1 (2.4 - 3.7)	3.0 (2.4 - 3.7)
Intervention	Yes	2.4 (1.9 - 2.9)	2.5 (2.0 - 3.0)	2.4 (1.9 - 2.9)
Sanidate	Yes	2.3 (1.7 - 2.9)	2.4 (1.9 - 3.0)	2.4 (1.9 - 3.0)
Virkon-S	Yes	3.0 (2.5 - 3.4)	3.1 (2.6 - 3.5)	3.0 (2.6 - 3.5)
Z-series	Yes	2.8 (2.3 - 3.3)	2.9 (2.4 - 3.4)	2.8 (2.3 - 3.3)

[a]Predicted means in top row of each cell, and the 95% confidence interval is in parenthesis under each predicted mean.
[b]Model estimates for EasyDECON DF-200 with no power washing were not reported due to viable spore counting errors.

Table 4: Log10 Viable Spore Reduction Estimates, Based on the Least Squares Fit Model, for *B. subtilis* Spores Inoculated on Porous Surfaces (Wool Fabric) and Treated with Disinfectants

Disinfectant	Power wash (yes or no)	One disinfectant application[a]	Two disinfectant applications	Three disinfectant applications
		Log10 reduction		
Clorox Conc.	No	0.6 (0.1 - 1.0)	1.3 (0.9 - 1.8)	1.7 (1.2 - 2.1)
Dioxiguard	No	0.5 (0.0)	1.2 (0.7 - 1.8)	1.6 (1.1 - 2.1)
ElectroBio	No	0.0 (-0.03 - 0.5)	0.8 (0.3 - 1.3)	1.1 (0.6 - 1.6)
Intervention	No	0.5 (0.0 - 1.1)	1.3 (0.7 - 1.8)	1.6 (1.1 - 2.1)
Sanidate	No	1.1 (0.6 - 1.6)	1.8 (1.3 - 2.3)	2.2 (1.7 - 2.7)
Virkon-S	No	1.0 (0.5 - 1.5)	1.8 ((1.2 - 2.3)	2.1 (1.6 - 2.6)
Z-series	No	0.7 (0.3 - 1.2)	1.5 (1.0 - 1.9)	1.8 (1.3 - 2.3)
Clorox Conc.	Yes	2.7 (2.1 - 3.3)	2.8 (2.2 - 3.4)	2.8 (2.2 - 3.3)
DF-200[b]	Yes	4.7 (4.2 - 5.2)	4.8 (4.3 - 5.3)	4.8 (4.3 - 5.2)
Dioxiguard	Yes	2.6 (2.0 - 3.1)	2.7 (2.2 - 3.2)	2.7 (2.1 - 3.2)
ElectroBio	Yes	2.3 (1.8 - 2.8)	2.4 (1.9 - 2.9)	2.3 (1.8 - 2.9)
Intervention	Yes	2.3 (1.8 - 2.9)	2.4 (1.9 - 3.0)	2.4 (1.9 - 2.9)
Sanidate	Yes	1.9 (1.3 - 2.4)	2.0 (1.4 - 2.5)	1.9 (1.4 - 2.5)
Virkon-S	Yes	2.7 (2.2 - 3.2)	2.8 (2.3 - 3.3)	2.8 (2.3 - 3.3)
Z-series	Yes	3.1 (2.6 - 3.6)	3.2 (2.7 - 3.7)	3.2 (2.6 - 3.7)

[a]Predicted means in top row of each cell, and the 95% confidence interval is in parenthesis under each predicted mean.
[b]Model estimates for EasyDECON DF-200 with no power washing were not reported due to viable spore counting errors.

follows: power washing, disinfectant type, number of disinfectant applications and sample surface (Table **2**). The decontamination treatment with the greatest sporicidal efficacy was power washing on porous surfaces, followed by three applications of EasyDECON DF-200, which resulted in a 4.8 log10 viable spore reduction (Table **3**). When disinfectants were applied alone and there was no power washing, log10 viable spores decreased after repeated applications for only Accel, Clorox Concentrate, and EasyDECON DF-200 disinfectants (Table **4**). When power washing was combined with disinfectant applications log10, viable spores were reduced after repeated applications of Accel, but log10 sporicidal activity increased for Clorox Concentrate and EasyDECON DF-200 disinfectants.

The log10 viable spore reduction for three disinfectants was compared with the equipment decontamination study conducted in 2016 (Table **5** and Figure **1**). The comparison shows analogous log10 viable spore reduction values for three independent

Table 5: Log10 Viable Spore Reduction, Based on Least Squares Fit Model, for Three Disinfectants Evaluated in 2016 and 2017 Equipment Decontamination Studies. The Disinfectants were Applied to Non-Porous Surfaces (Steel Washers) Inoculated with *B. subtilis* Spores using a Single Spray Application

Disinfectant type	2016 First study – predicted log10 reduction	2016 Second study - predicted log10 reduction	2017 predicted log10 reduction
EasyDECON DF-200	1.42	2.03	na
ElectroBiocide	0.68	1.11	0.7
Virkon-S	1.14	1.05	1.3

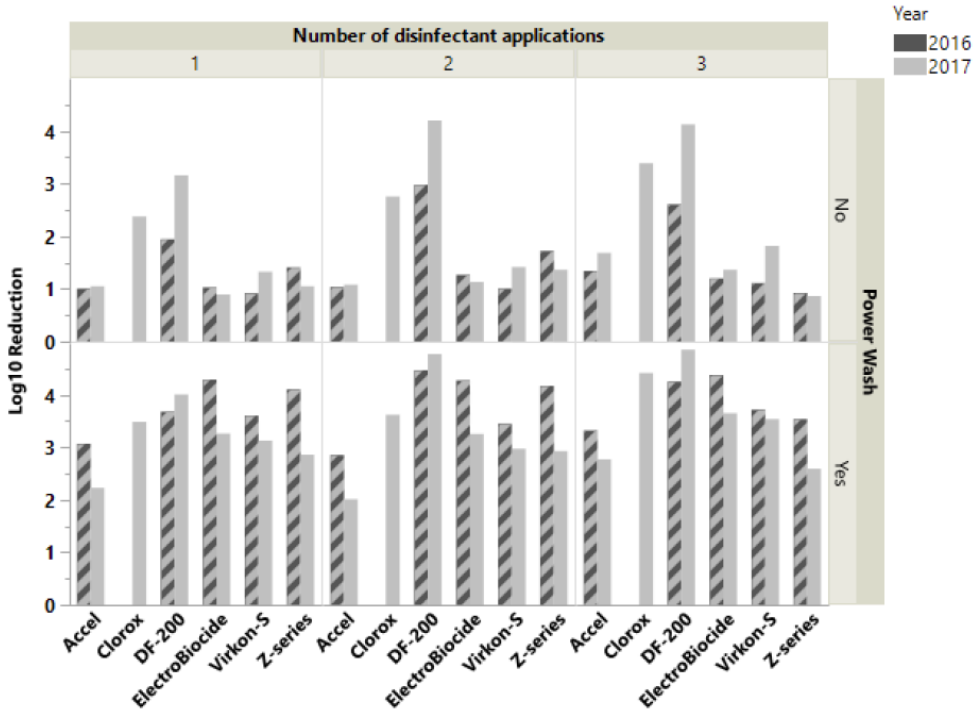

Figure 1: Comparison of two-stage decontamination field studies conducted in 2016 and 2017. The average log10 reduction is graphed by disinfectants (lower x-axis) number of disinfectant applications (upper x-axis), power washing (right y-axis) and by year (legend).

and separate disinfectant tests, when conducted on non-porous surfaces with a single spray application of a disinfectant.

4. DISCUSSION AND CONCLUSIONS

The goal of this study was to evaluate the ability of a mobile power washer and disinfectant treatments to decontaminate agricultural equipment. Power washing and type of disinfectant increased log10 sporicidal efficacy to the greatest degree, with the number of disinfectant applications and sample type being less effective for improving sporicidal activity. The results from this study substantiate previous research that concluded that power washing is a crucial component in the decontamination of field equipment [2-6]. Power washing is well suited for field equipment, vehicles, storage facilities and cargo containers, but may not be well suited for sensitive or electronic equipment.

The ORP of the eight disinfectants ranged from 323 to 1,092 mV (Table **1**). Setlow [13] reported that oxidant disinfectants with ORP values ranging from 650 to 700 mV inactivated *E. coli* and *Salmonella* within a few seconds. Our study found that there was no relationship between ORP value and log10 reduction of viable spores for oxidant disinfectants applied alone, i.e. without power washing (p-value = 0.39). The diluted concentration of the disinfectants ranged from 160 to 54,782 ppm (Table **1**). There was no relationship between disinfectant concentration and log10 reduction if the DF-200 data was excluded (p-value = 0.09454). The DF-200 concentration was high compared to the

other disinfectants; therefore, it skewed the regression analysis and resulted in a relationship between disinfectant concentration and the log10 viable spore reduction across all disinfectants. The poor correlation between oxidant disinfectant properties and sporicidal efficacy suggests that other factors besides concentration or ORP affected the overall efficacy of the disinfectants tested.

The two sample surfaces represented porous (wool fabric) and non-porous (steel washer) surfaces. The study hypothesis for testing the two sample surfaces was that spores would be more deeply embedded into the wool fabric as opposed to drying on the surface of steel washers; therefore the decontamination treatments should have been less effective for the porous surfaces. Analysis of the log10 viable spore reduction data showed that the sample surface had the least impact on disinfectant efficacy. These results imply that power washing and disinfectant treatments were able to either dislodge or inactivate the *B. subtilis* spores similarly for porous or non-porous surfaces [5,6].

Power washing increased the log10 viable spore reduction by an average of 55% for both non-porous and porous surfaces when comparing two-stage treatments with disinfectants applied alone. This observation was similar across all disinfectants and repeat applications (Tables **3** and **4**). In the 2016 study, which was a similar two-stage decontamination process, power washing was 217% more effective in reducing viable spores. It is unclear why power washing had such a large discrepancy in spore efficacy between the two study years. Guan *et al*. [4] evaluated two-stage decontamination of field equipment and found that power washing increased the log10 reduction of *Geobacillus stearothrmophilis* spores by 95% in comparison to no power washing treatments. Their results are somewhat comparable to the power washing results in this study. Mobile power washing systems like the mobile system used in this study can remove up to 90% of soil from field equipment [14].

These results validate that *B. subtills* spore inactivation by disinfectants applied alone were consistent and accurate, when tested across multiple studies over two years (Figure **1**). Further research is still needed, especially for large agricultural producers that need stationary decontamination stations to sanitize transport vehicles entering their properties. Also, the decontamination of sea containers and commodity transport containers is crucial to protecting international agricultural trade. Research is also needed for power washing parameters such as nozzle pressure and distance from surfaces, pre-washing systems to remove road grime, and sprayer water recycling systems. With reoccurring epidemics that threaten to spread zoonotic diseases associated with agricultural commodities, it is becoming a national priority to advance more effective and efficient decontamination technologies.

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